Applying Semiconductor Technologies and Metrology Tools to Biomedical Research:

Manipulation and Detection of Single Molecules

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intel.

Talk Overview

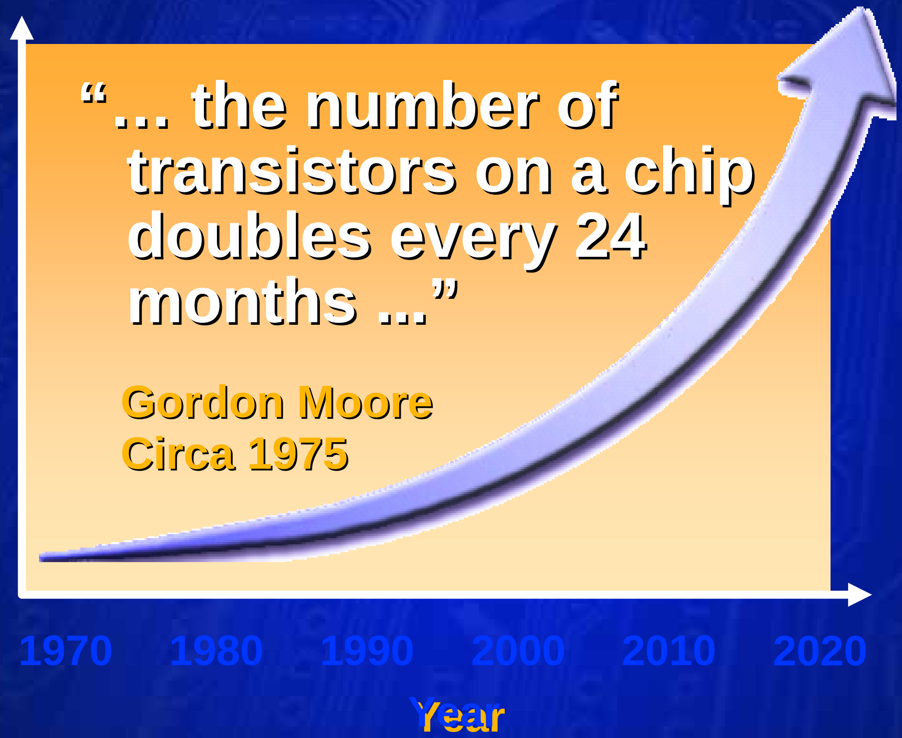
- Introduction
- Focused ion beams → Microfluidics
  - Transport of ultra-low concentration mixtures
    - 3-D Hydrodynamic Focusing
- Ultra-sensitive Detection via Raman spectroscopy

What Does Molecular Diagnostics Need to Succeed?

- Ability to distinguish diseased organisms from healthy organisms
- Diagnose disease and select best treatment at early stages
- Ability to make sense of 100,000+ biomolecules in a complex mixture

Technology Trajectory

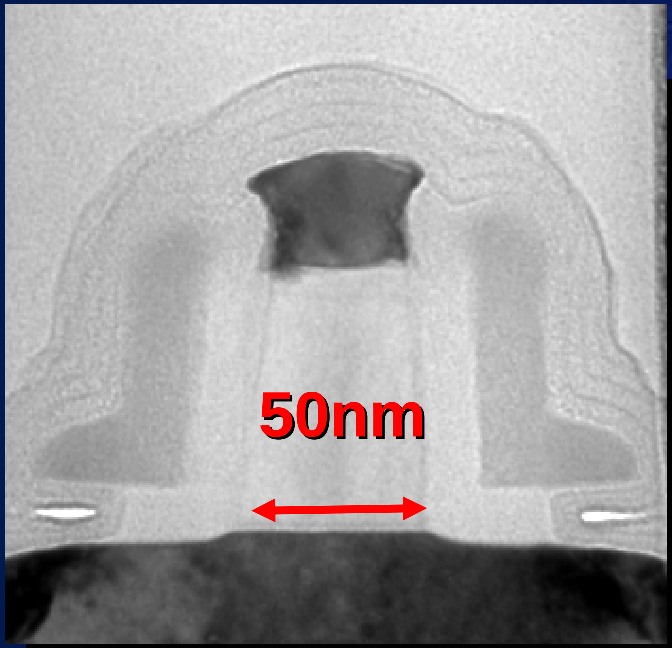
Moore's Law



Extending and Expanding Moore's Law

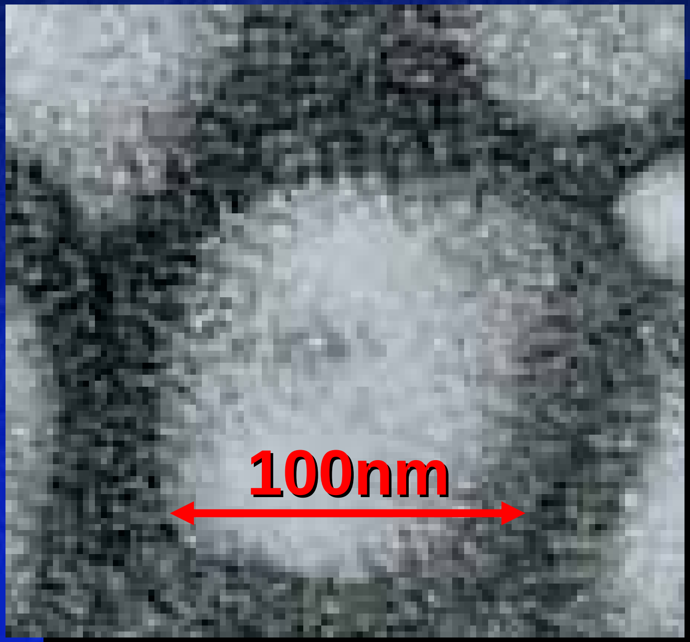
Current Status of Silicon

50 nm transistor dimension is ~2000x smaller than diameter of human hair



Transistor for 90nm Process

Source: Intel



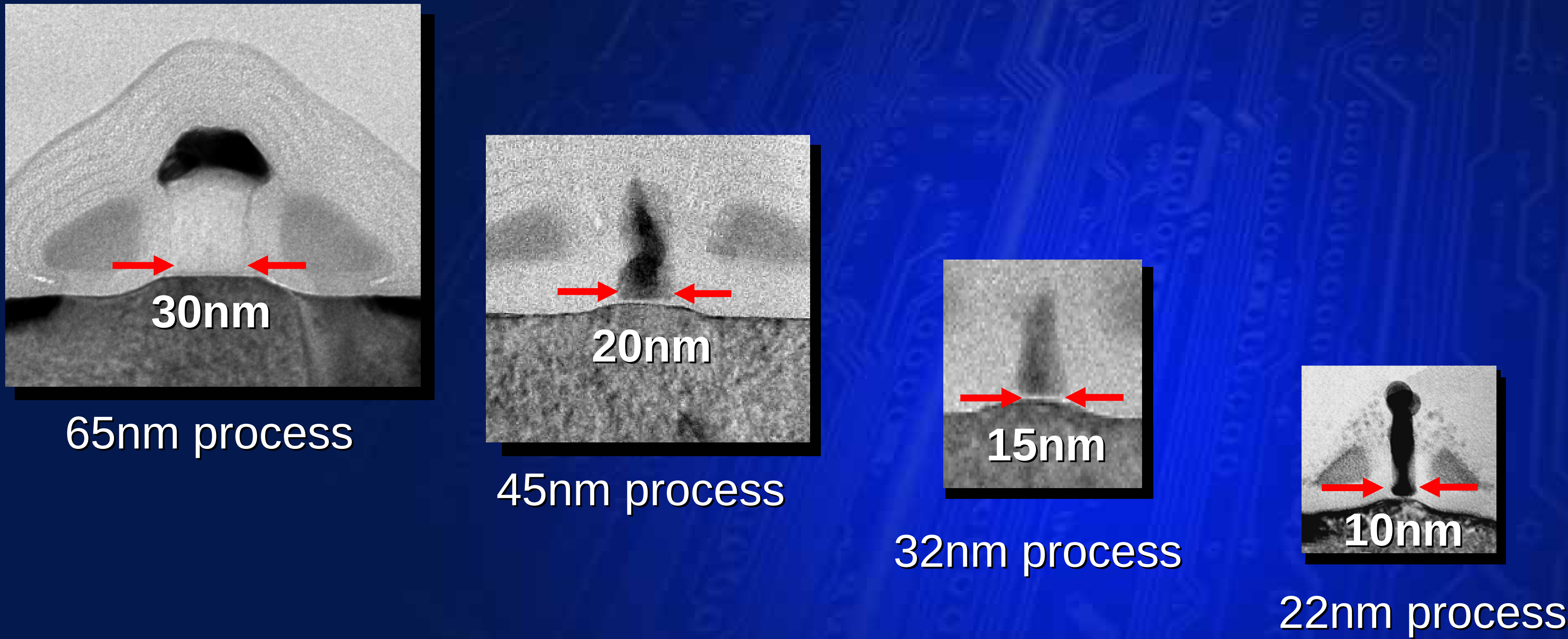
Influenza virus

Source: CDC

Gate dielectric thickness = 1.2nm
Nanotechnology in High Volume Production

Intel's Transistor Research Down to 10nm

Experimental transistors for future process generations



We are investigating several options at <10nm

Source: Intel

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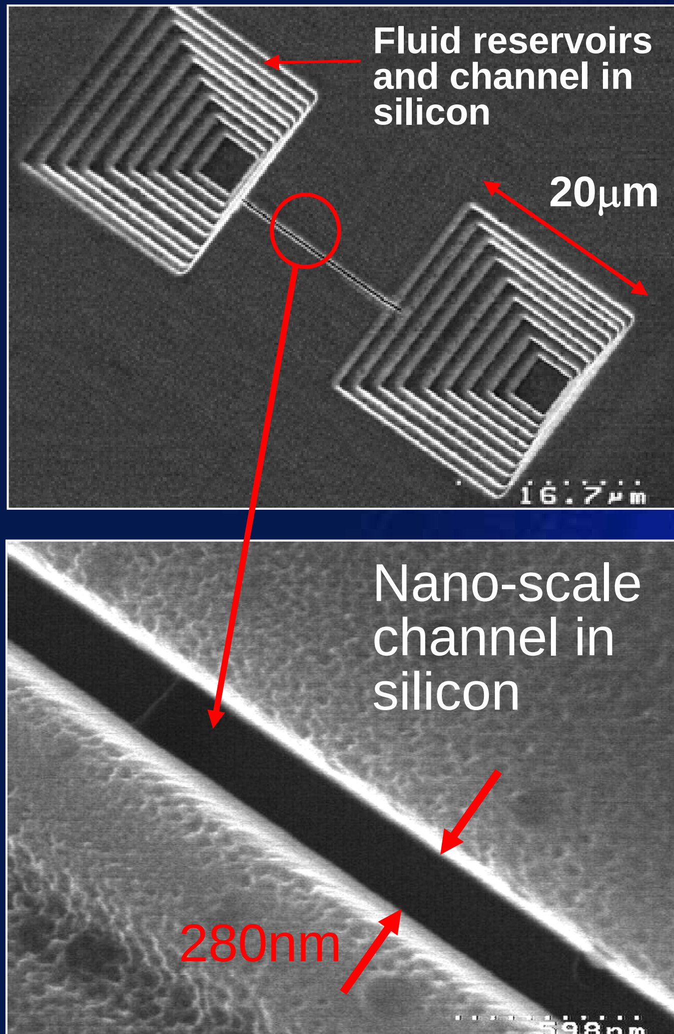
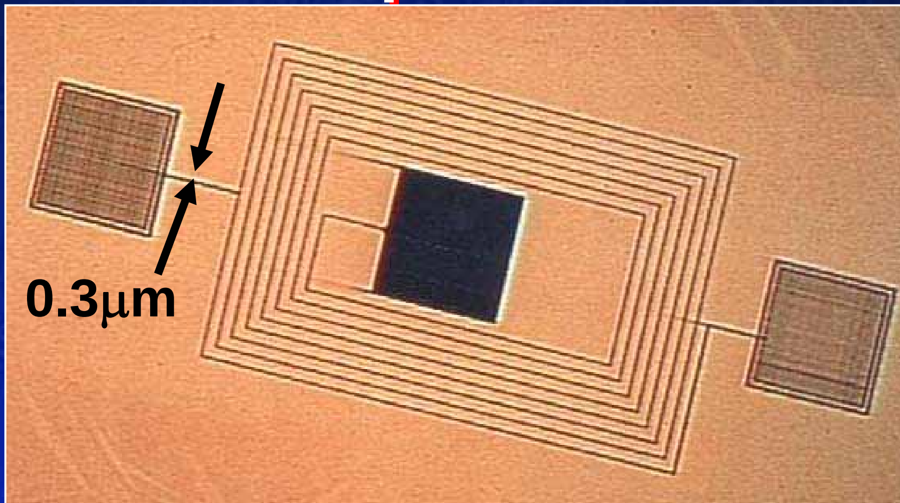
Raman spectrometer with a chip: Microfluidic Transport

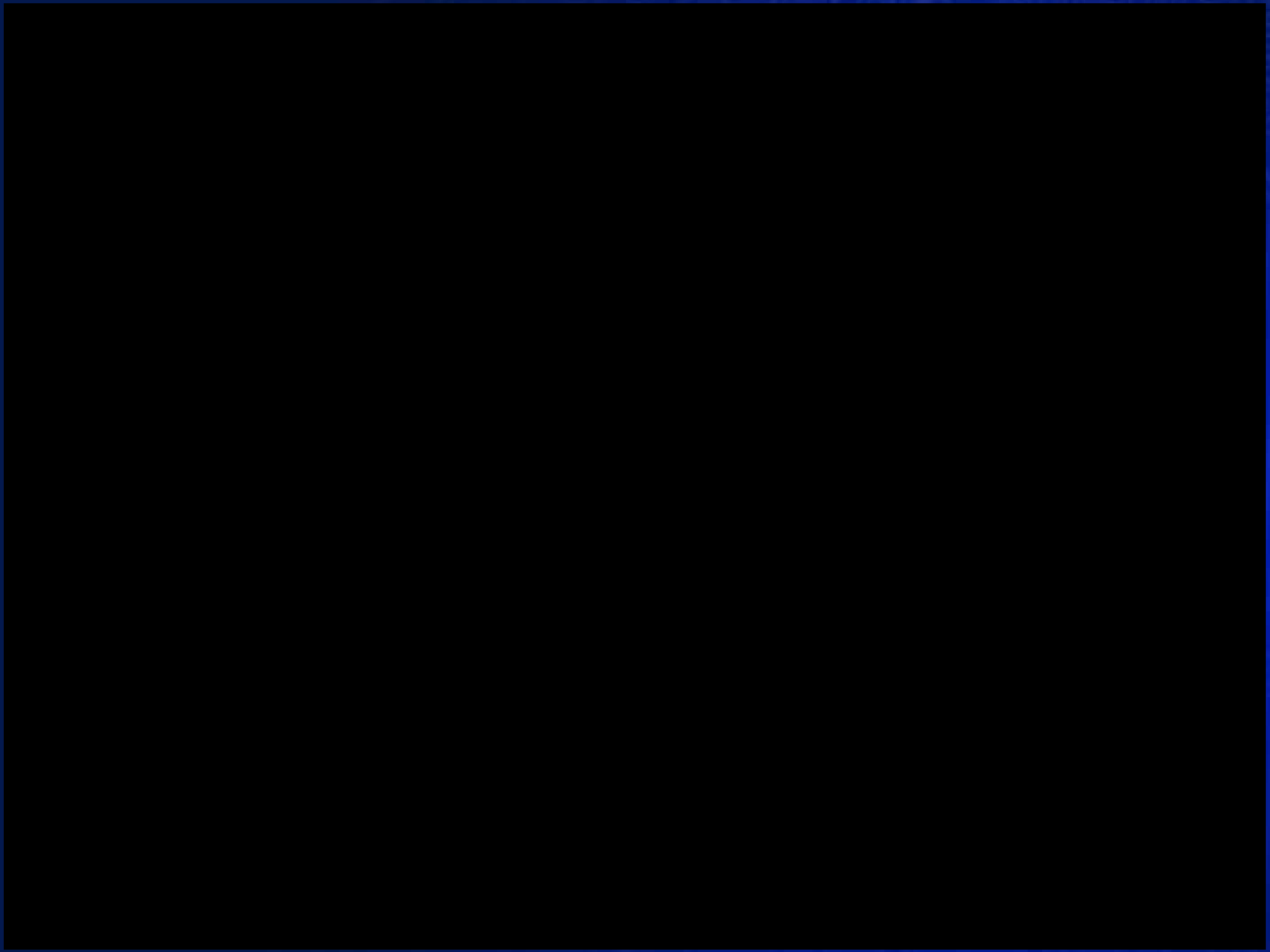




- Complex micro-fluidic structures made with Focused Ion Beam (FIB) tools
  - Rapid prototyping enables quick design & fabrication of bio-chips
- FIB technology for chip diagnostics now being also used for MEMS prototyping:
  - Excellent synergy between MEMS research and other technology labs in Intel

Manipulating at the Nanoscale

Laser used to manipulate DNA attached to beads



‘DNA sling shot’

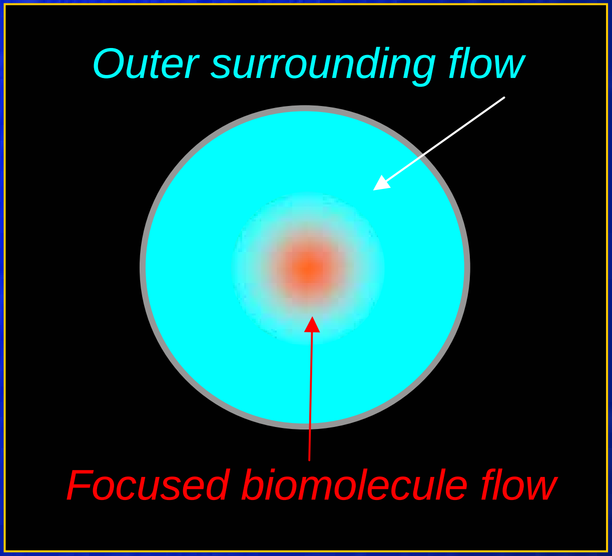
Advanced Microfluidics

3-D 'Hydrodynamic Focusing'

Applications: Study and sort small cells and biological molecules, single molecule detection, protein isolation

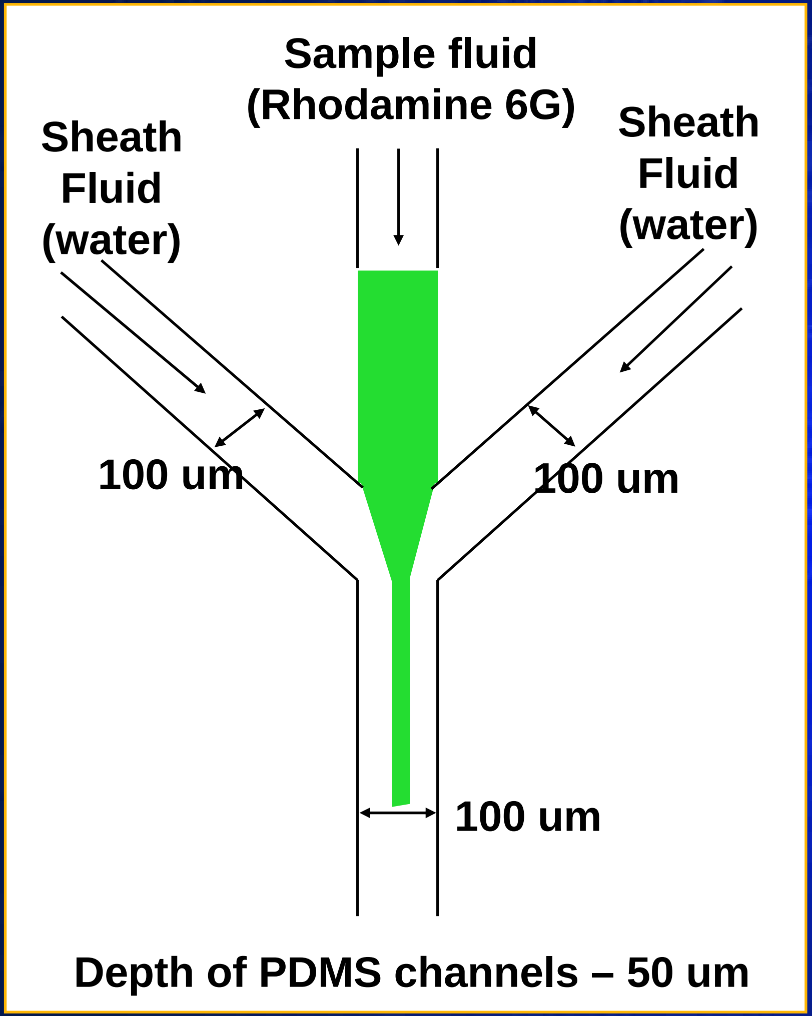
Advantages:

- Precision positioning of molecules for optical detection
- Absolute isolation of the sample from the channel wall surface
- Control of the focused stream over the third (z-direction) dimension
- Reduced sample volume that is comparable and adjustable to the detection volume

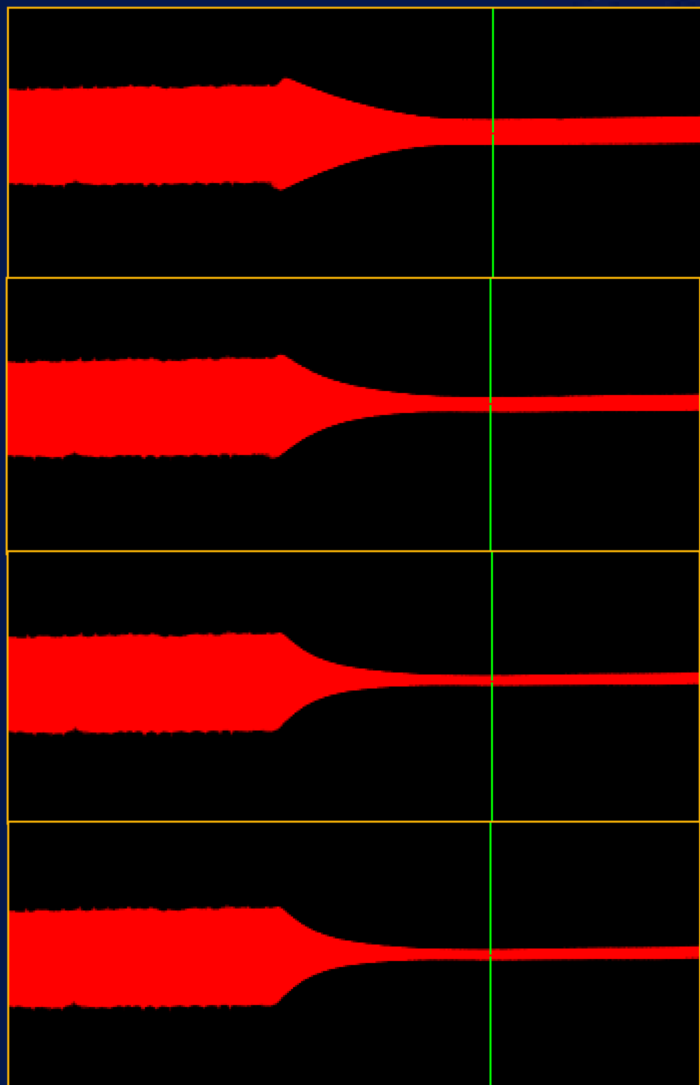


Cross-sectional view of a microfluidic channel

Two-dimensional Hydrodynamic Focusing



Two-dimensional Hydrodynamic Focusing Results



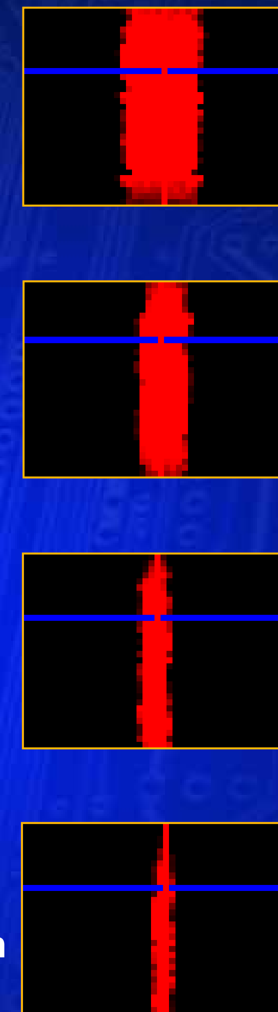
Top-view

Sheath flowrate = 10 ul/min
Sample flowrate = 10 ul/min

Sheath flowrate = 10 ul/min
Sample flowrate = 5 ul/min

Sheath flowrate = 10 ul/min
Sample flowrate = 2.5 ul/min

Sheath flowrate = 10 ul/min
Sample flowrate = 1.25 ul/min

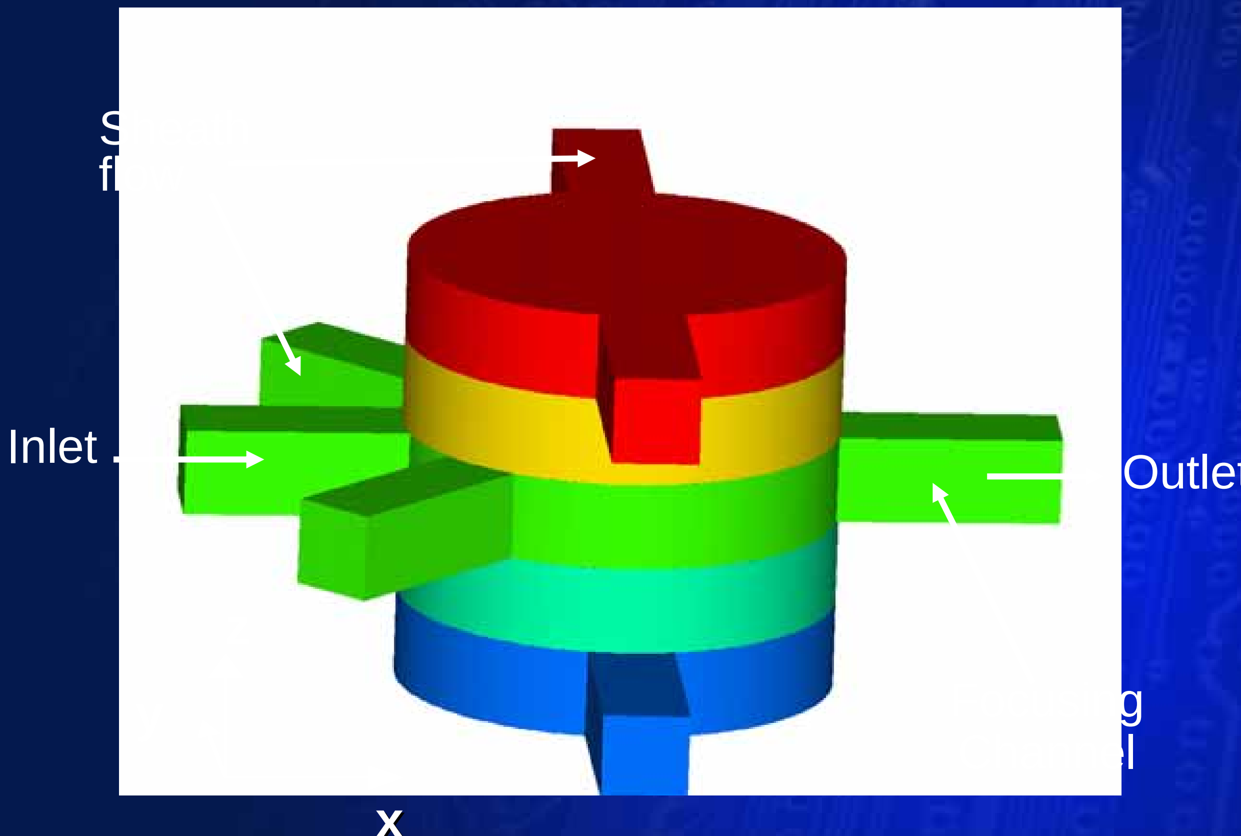


Cross-section

Effect of varying sample flow rates on the focusing width

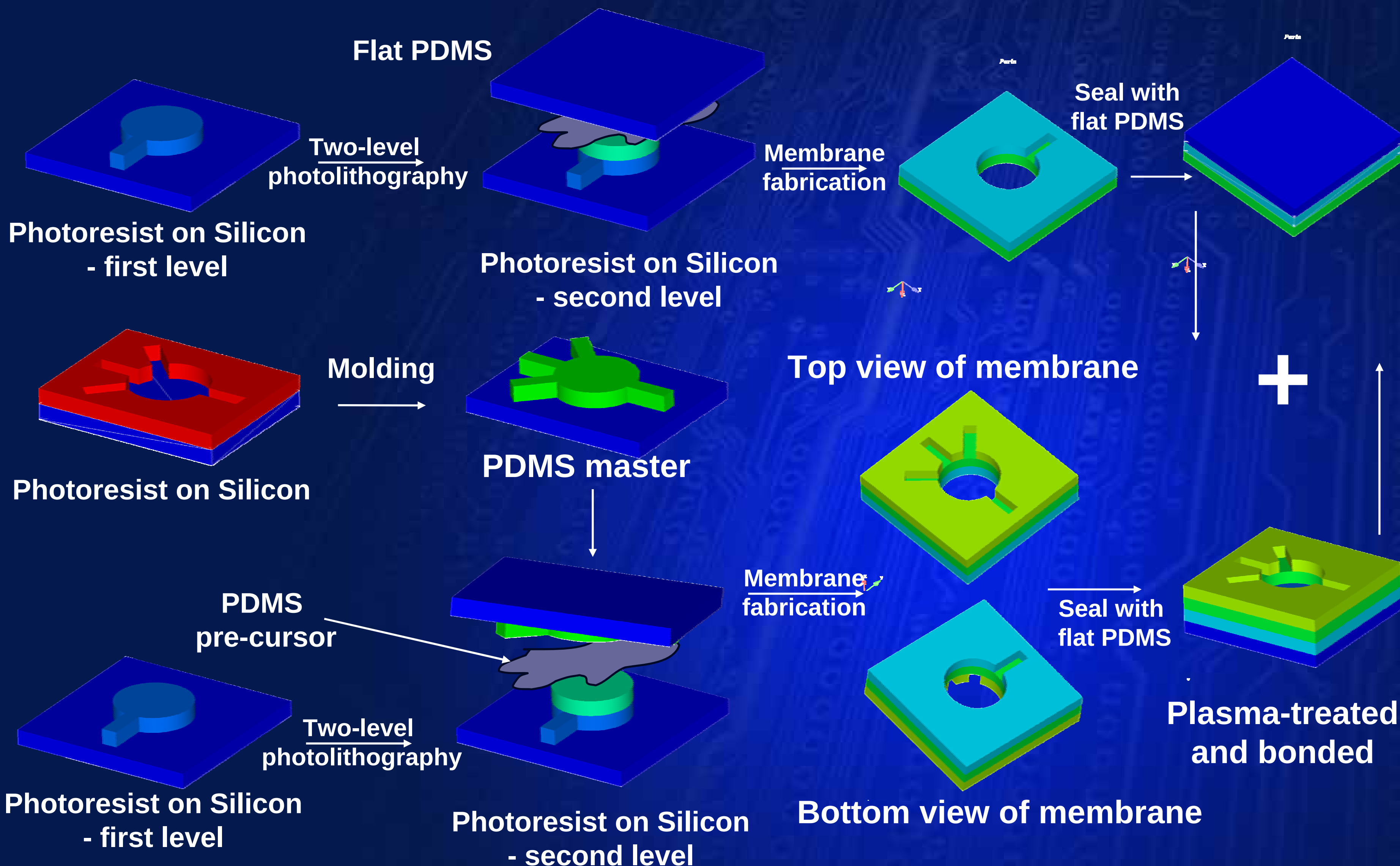
Advanced Microfluidics

3-D ‘Hydrodynamic Focusing’



* *N. Sundararajan et al., Nanotech 2002.*

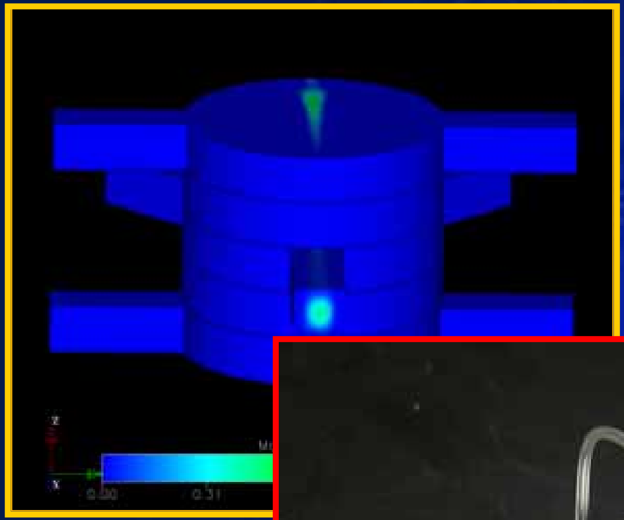
Fabrication of 3-D Hydrodynamic Focusing Chip*

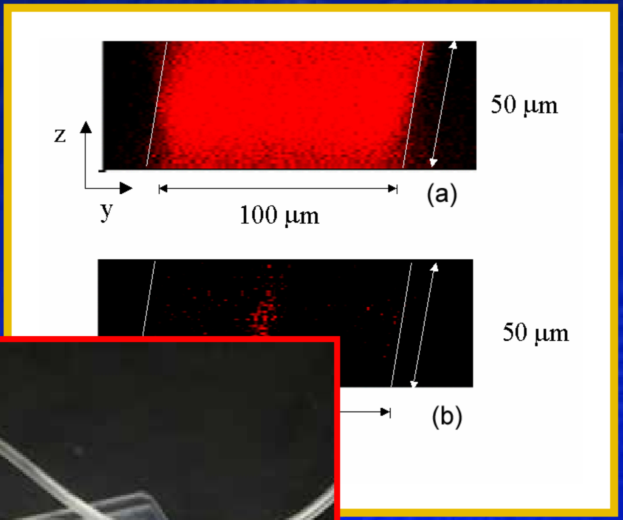


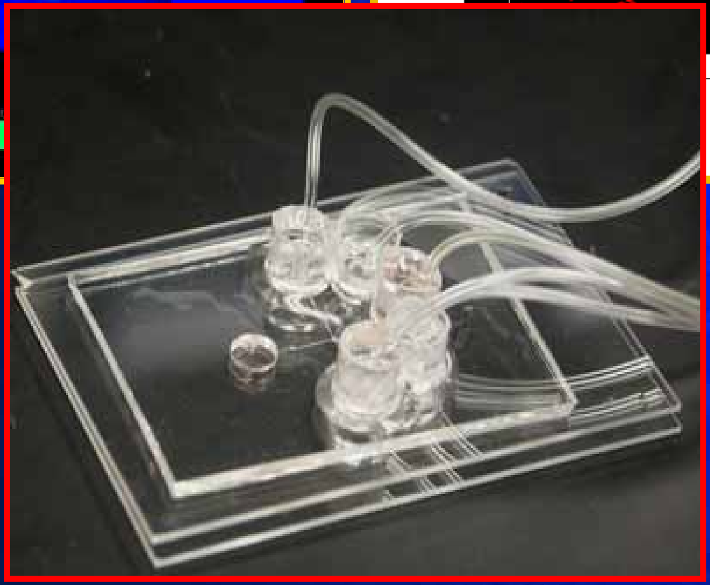
* Membrane sandwich method from Whitesides group

Advanced Microfluidics

3-D 'Hydrodynamic Focusing'

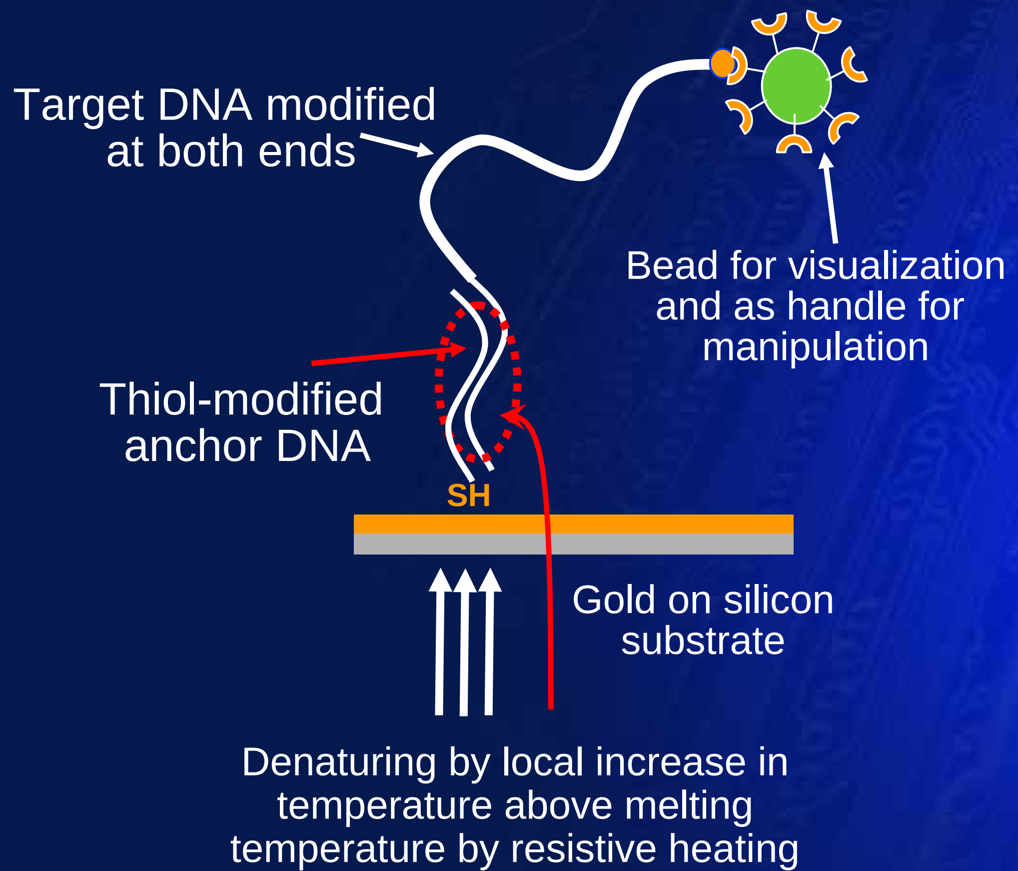






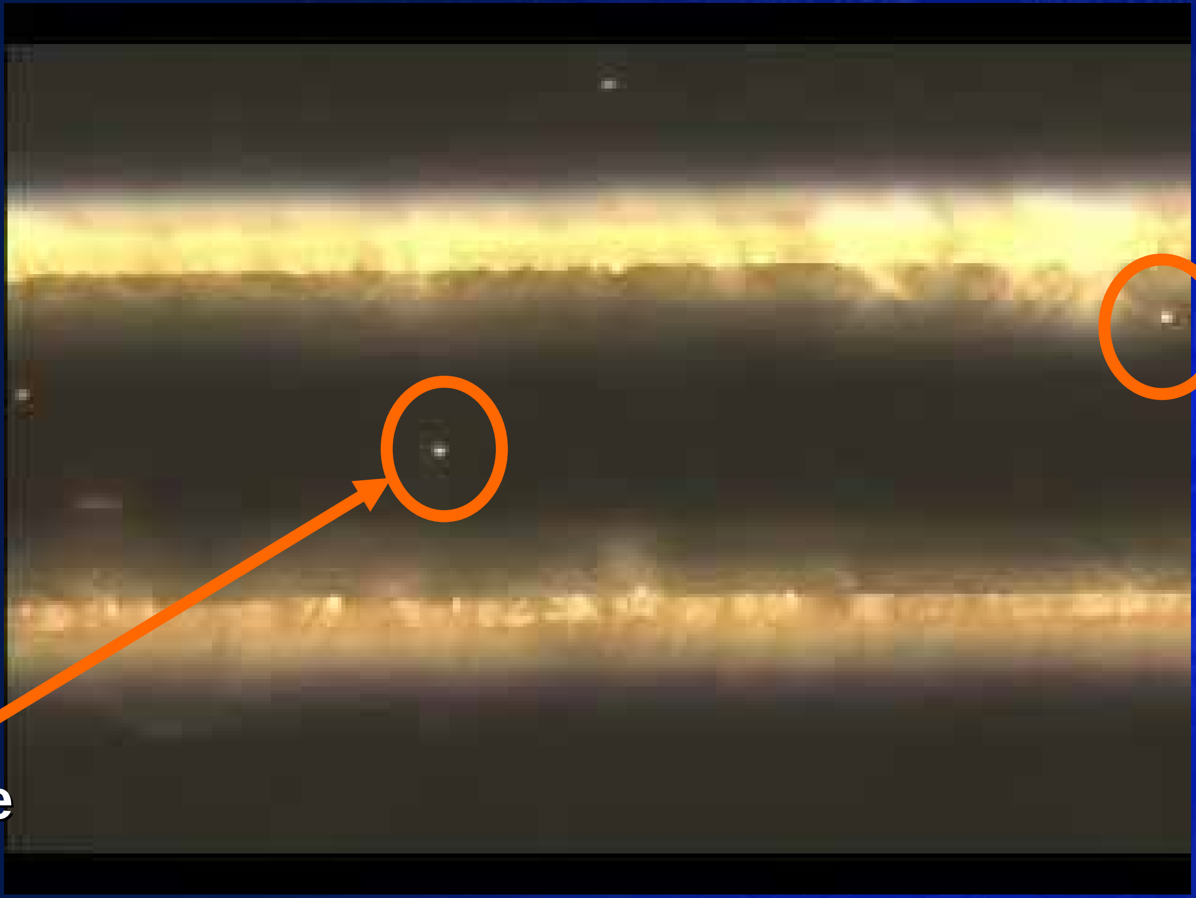
** N. Sundararajan et al., Nanotech 2002.*

Single Molecule Isolation & Manipulation: Our Approach



- High throughput – multiple single molecules isolated simultaneously
- Specific target molecules isolated by tailoring the anchor DNA and end-modification (multiplexity)
- Controlled release through electrical heating
- Selectivity of release achieved through selection of melting point of anchor DNA sequence

Video of Single Molecule Dispenser



1st molecule release

2nd molecule release

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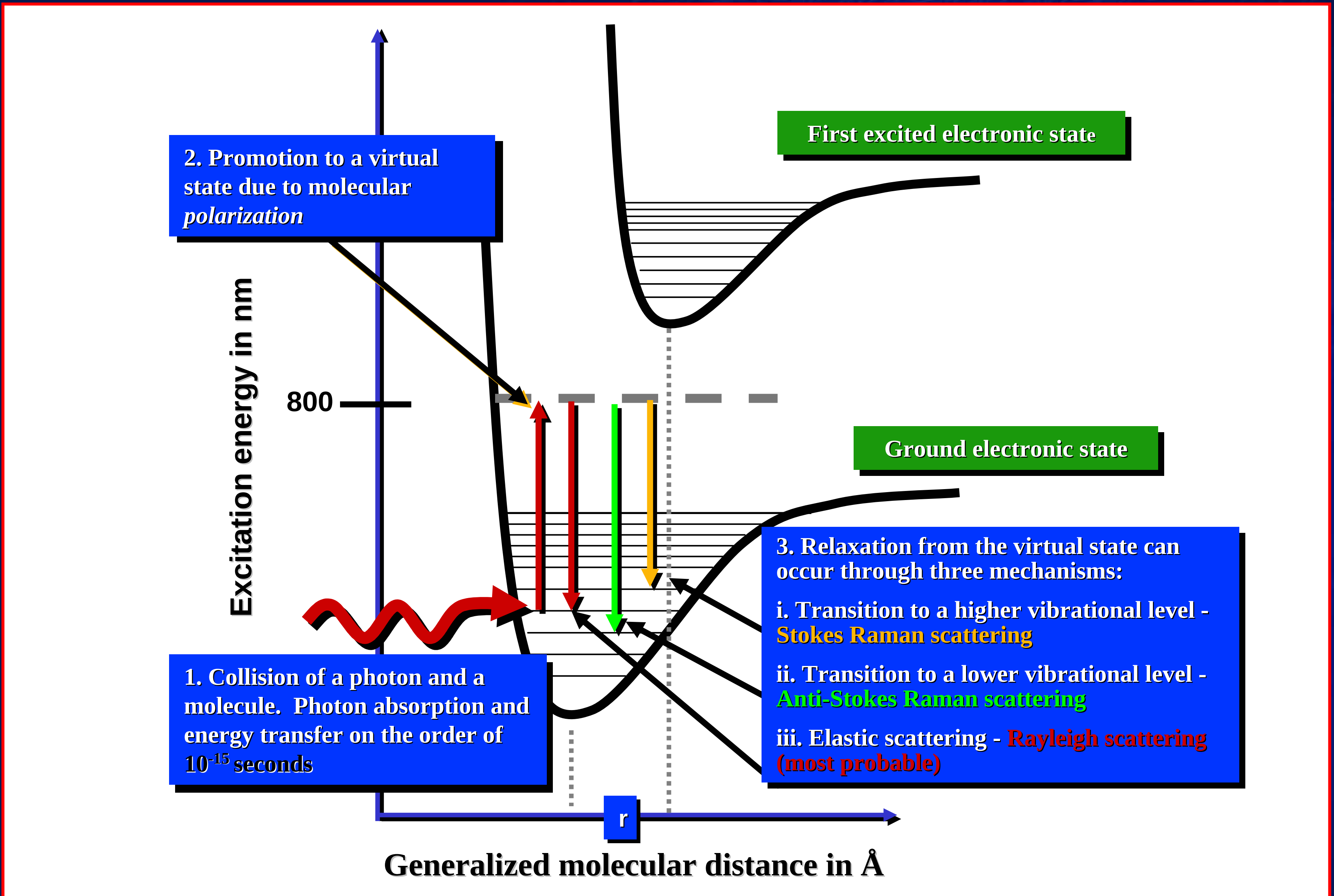
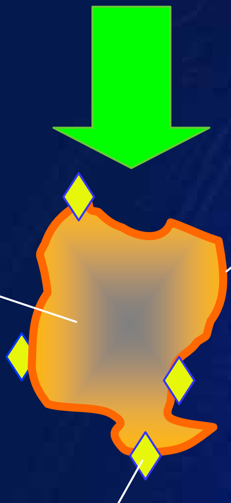
- Ultra-sensitive Detection via Raman spectroscopy
  - Label-free detection of single molecules
  - Label-based detection
    - New type of Raman-coded probe

Raman Spectroscopy



Surface Enhanced Raman Scattering (SERS)



EM: enhanced optical fields due to excitation of electromagnetic resonances in the metal ("global effect").

CE: electron-mediated resonance Raman effect via a charge transfer intermediate state ("local effect").

J. A. Creighton et al. *J. Chem. Soc. Faraday Trans. 2* 1979, 75, 790-798

A. Otto, in *Light Scattering in Solids,* edited by M. Cardona and G. Guentherodt (Springer, Berlin, 1984), p. 289.

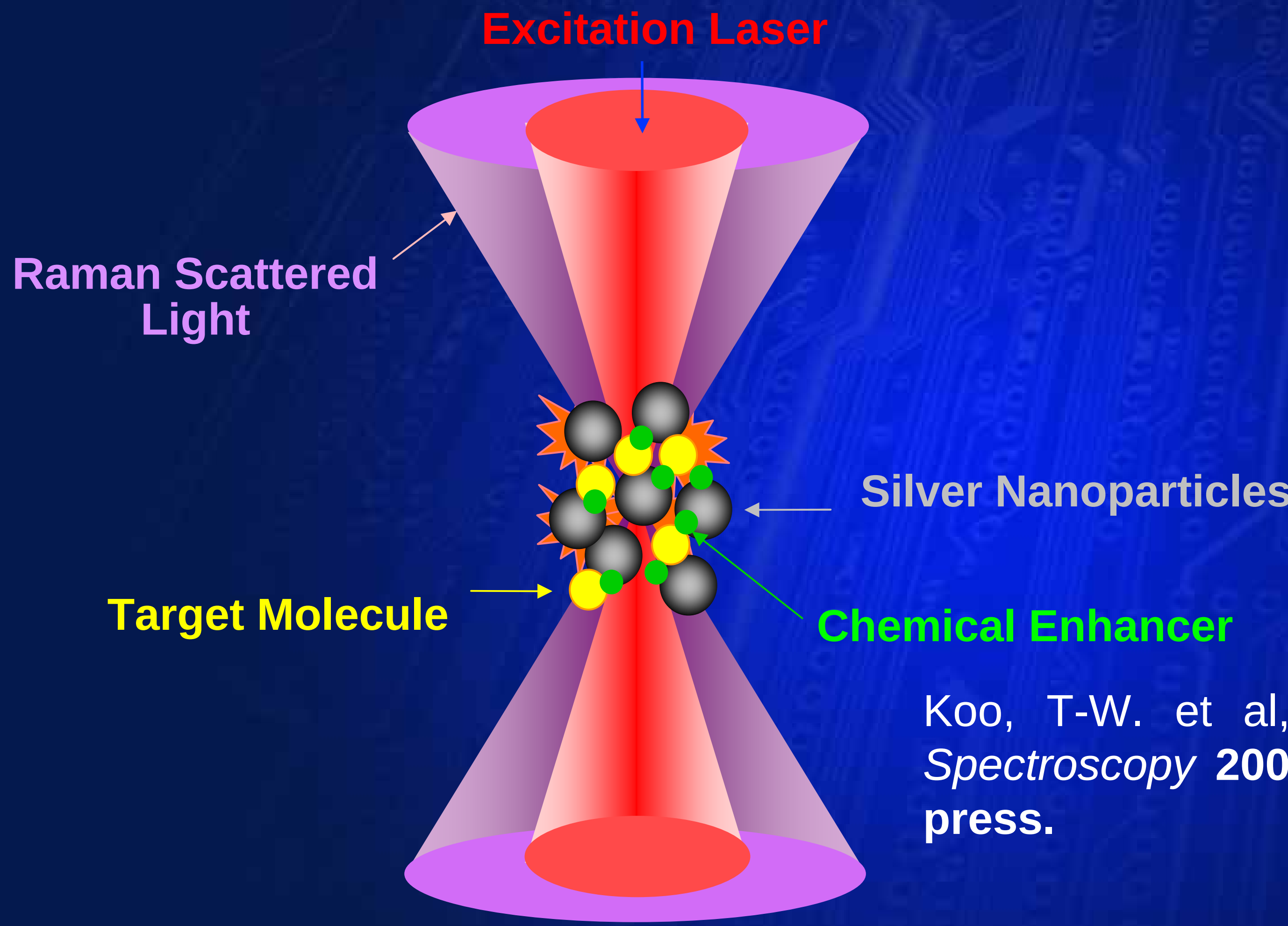
M. Moskovits, Rev. Mod. Phys. **57**, 783 (1985).

Kneipp, K.; et al *Phys. Rev. Lett.* 1997, 78, 1667-1670.

Nie, S.; Emory, S. R. *Science* 1997, 275, 1102-1106.

Xu, H.; Bjerneld, E. J.; Käll, M.; Börjesson, L. *Phys. Rev. Lett*. 1999, 83, 4357–4360.

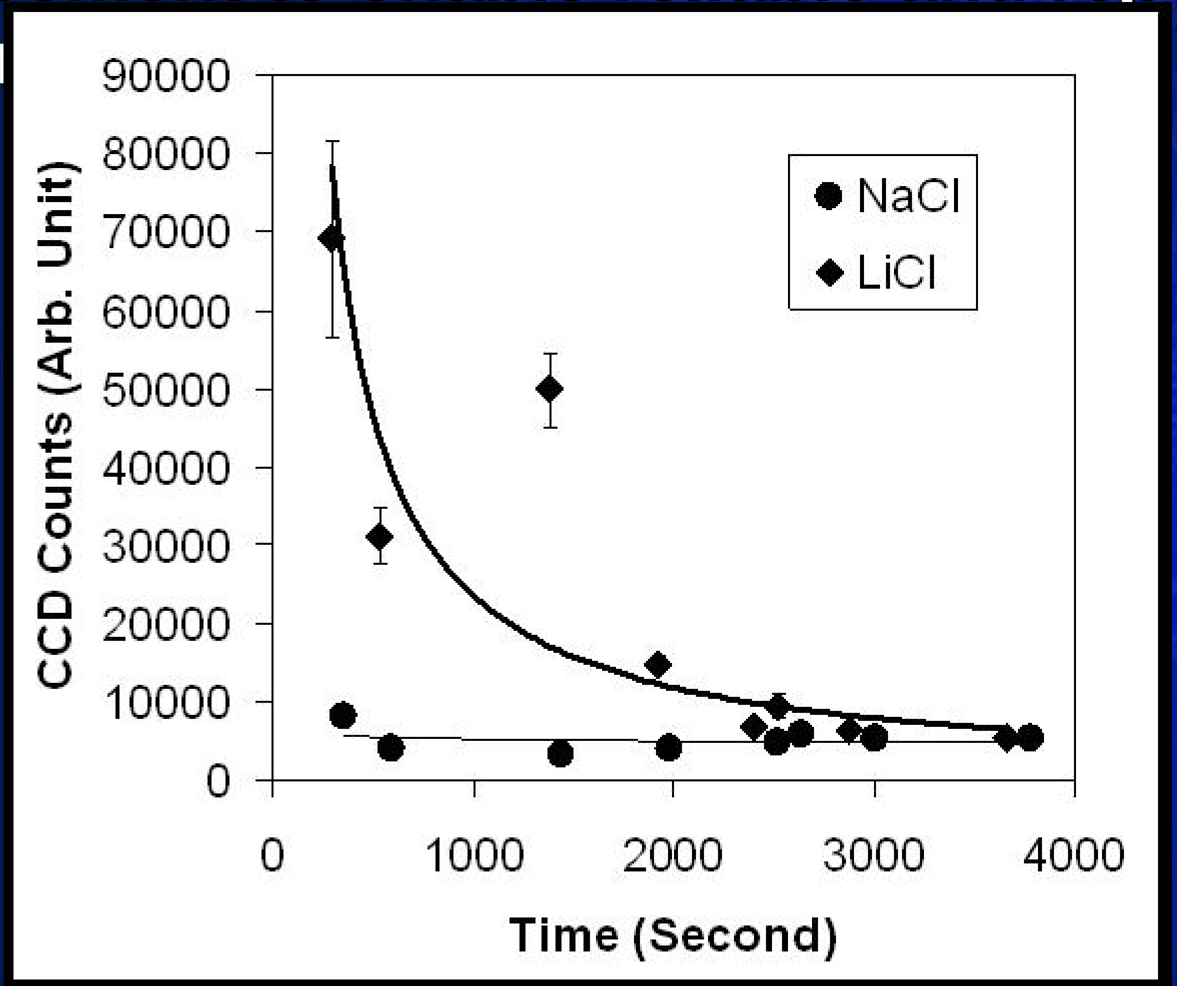
Metallic Nanoparticle Substrates for SERS



Koo, T-W. et al, *Applied Spectroscopy* **2004, 58, in press.**

Chemical Enhancement

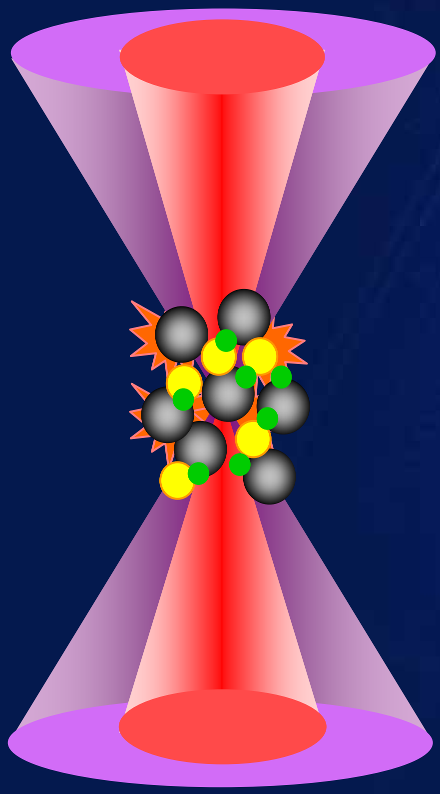
- **Cation selection is key to additional enhancement to enable reliable and repeatable single r**

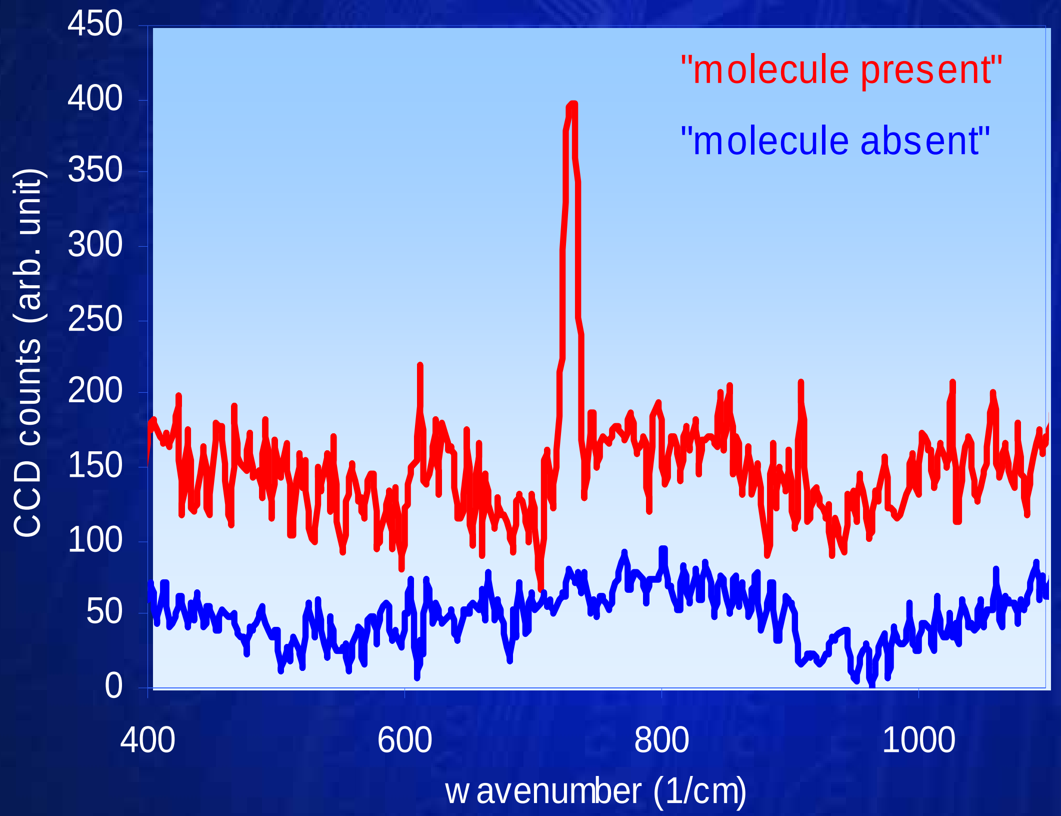


Height of 1510 cm^{-1} peak of Rhodamine 6G

Single Molecule Detection

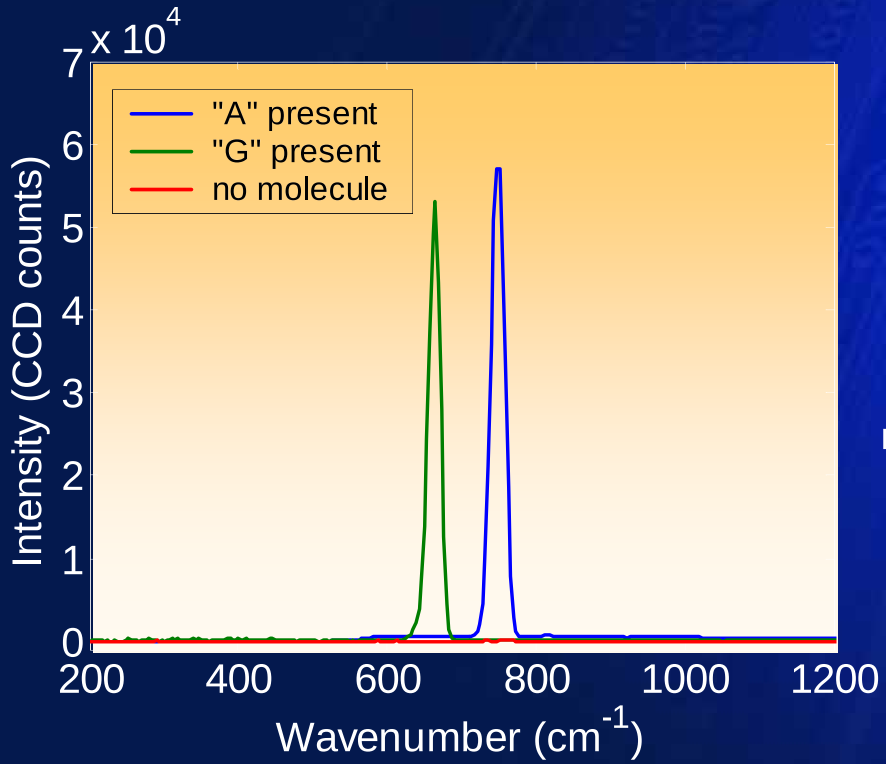
Statistical fluctuation and quantized peak intensity indicates detection of single adenine molecule

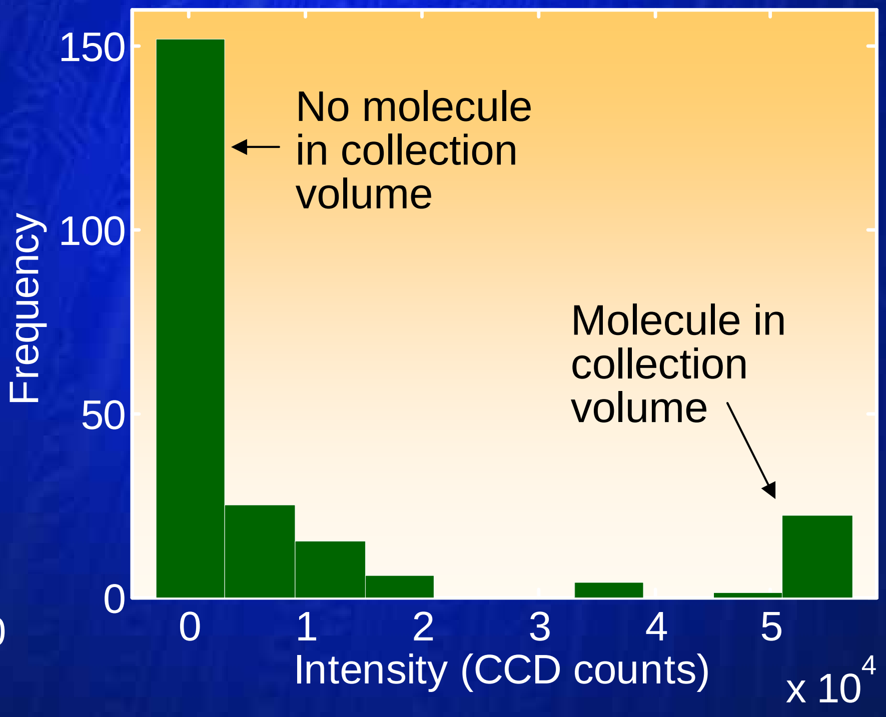




Single Nucleotide Detection by Multi-Photon Spectroscopy

- Unique signals observed from single molecule concentration of dAMP and dGMP nucleotides
- Signal "blinking" was observed due to molecular diffusion into and out of collection volume → quantized intensity observed





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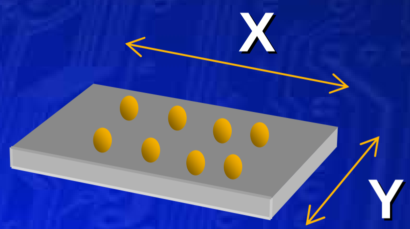
Multiplex Detection Systems

Physical-size encoded identifiers

<u>Micro-arrays</u>

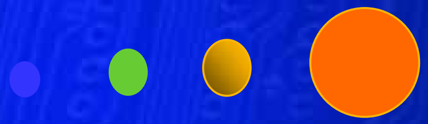
X-Y coordinators



Feature size: > 1 micron

<u>Quantum dots</u>

fluorescence wavelengths



1nm to 100 nm

<u>Metal bars</u>

positions of different metals

Sub-microns

Composite Organic-Inorganic Nanoparticles (COINS)

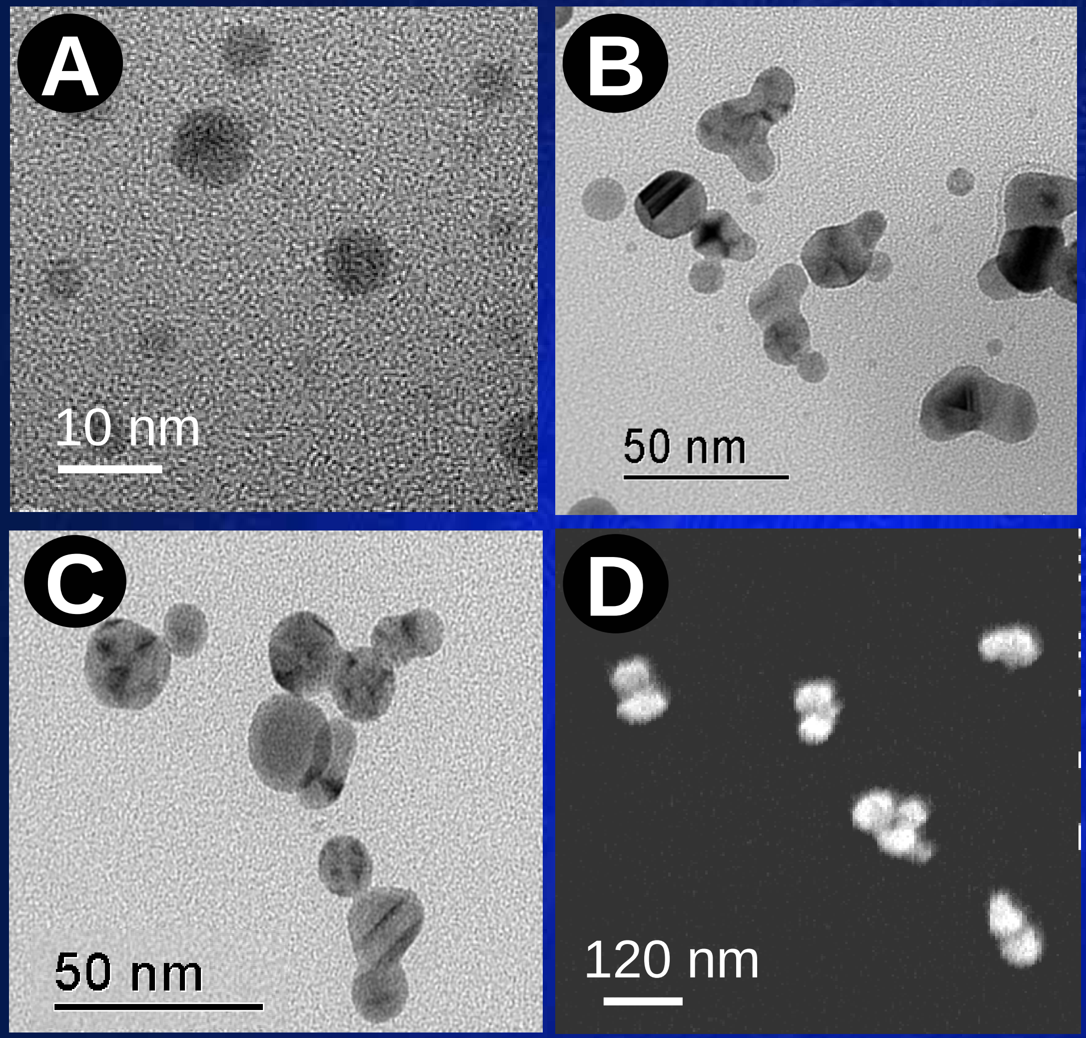
COINs have the ideal features

- Label "glue" silver particles
- Nanoparticles protect Raman labels

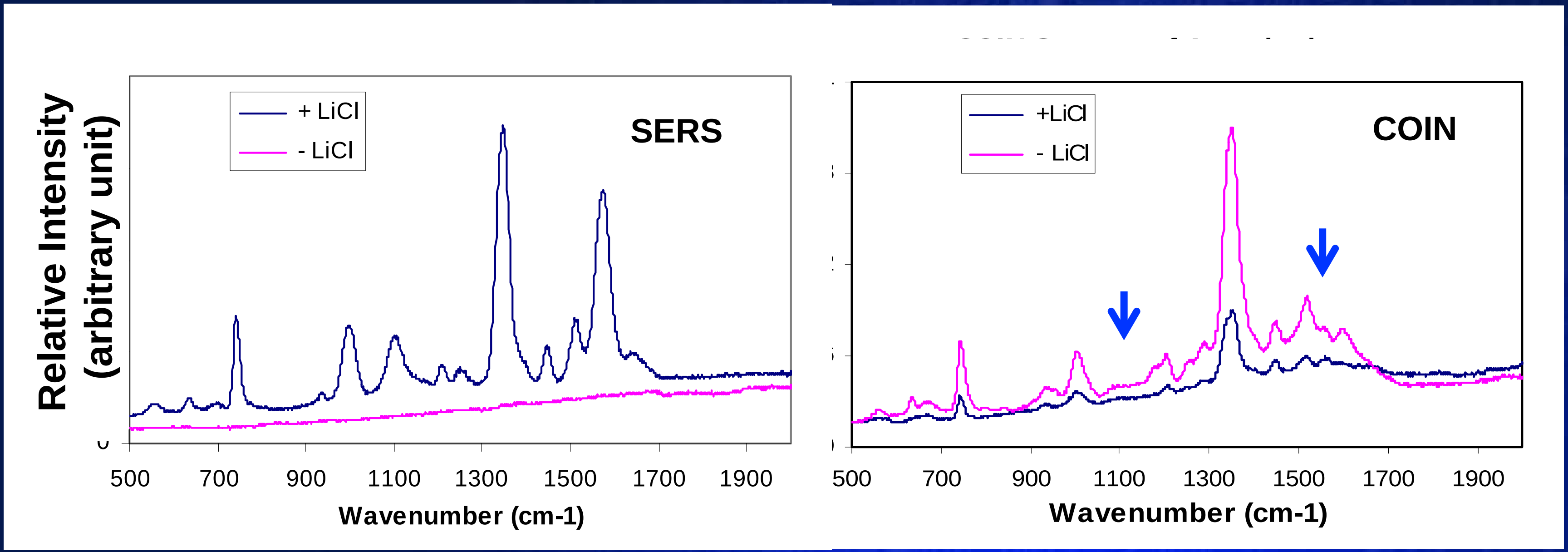
Key issues

- Size control
- Structure stabilization

COINs Are Metal Clusters

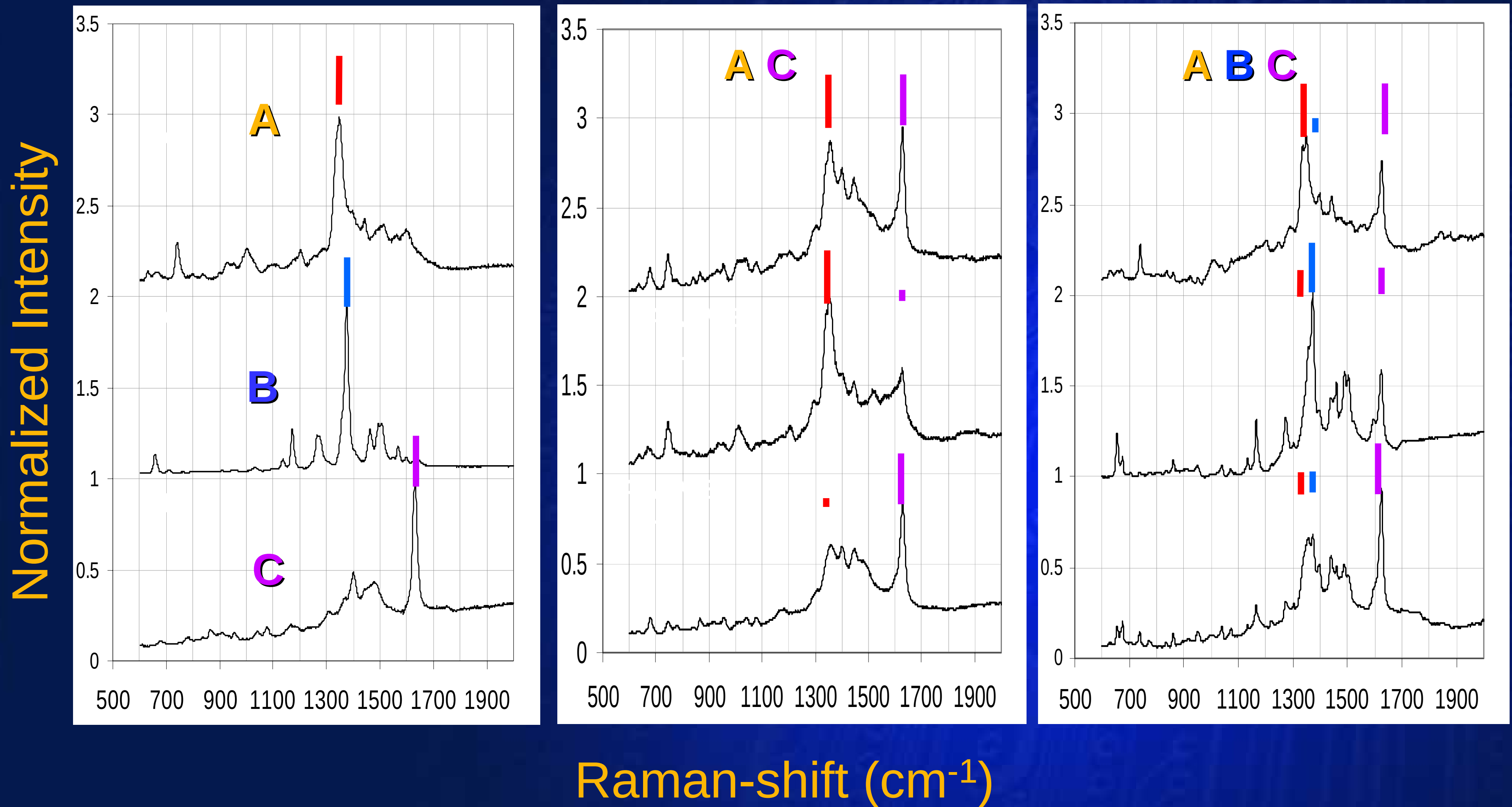


COINs Have Intrinsic and SERS-like Signals

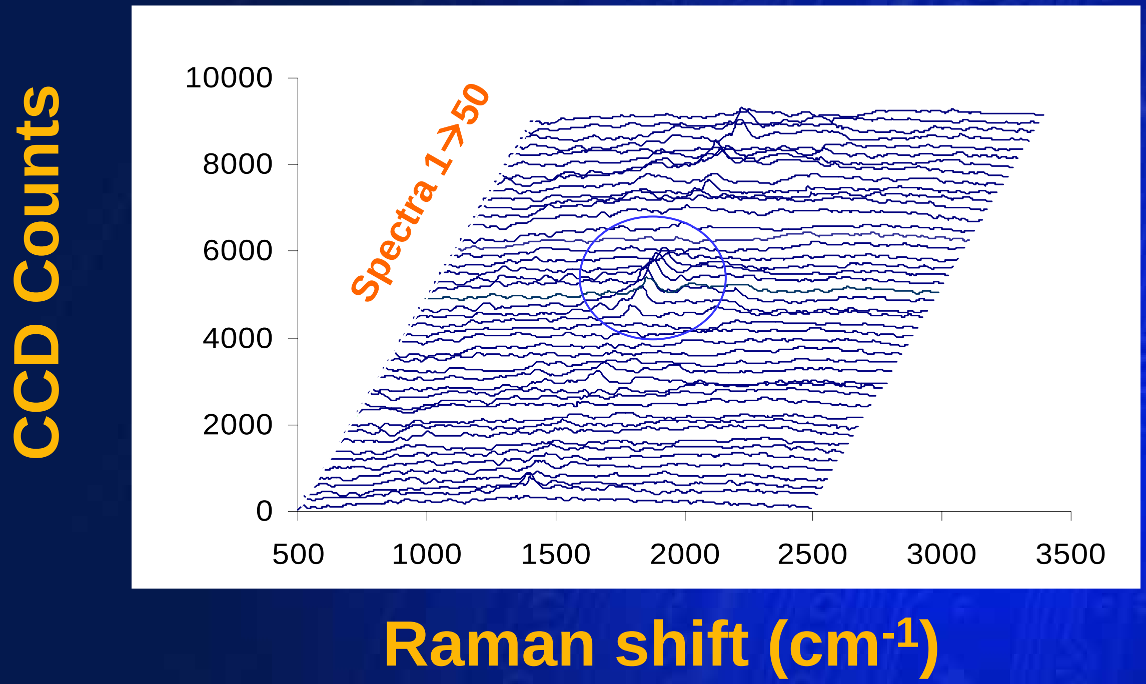


	SERS of Raman label A	COIN of Raman label A
Salt-induced Aggregation	Yes	No
Spectra	Complex	Simple

Examples of COIN Signatures

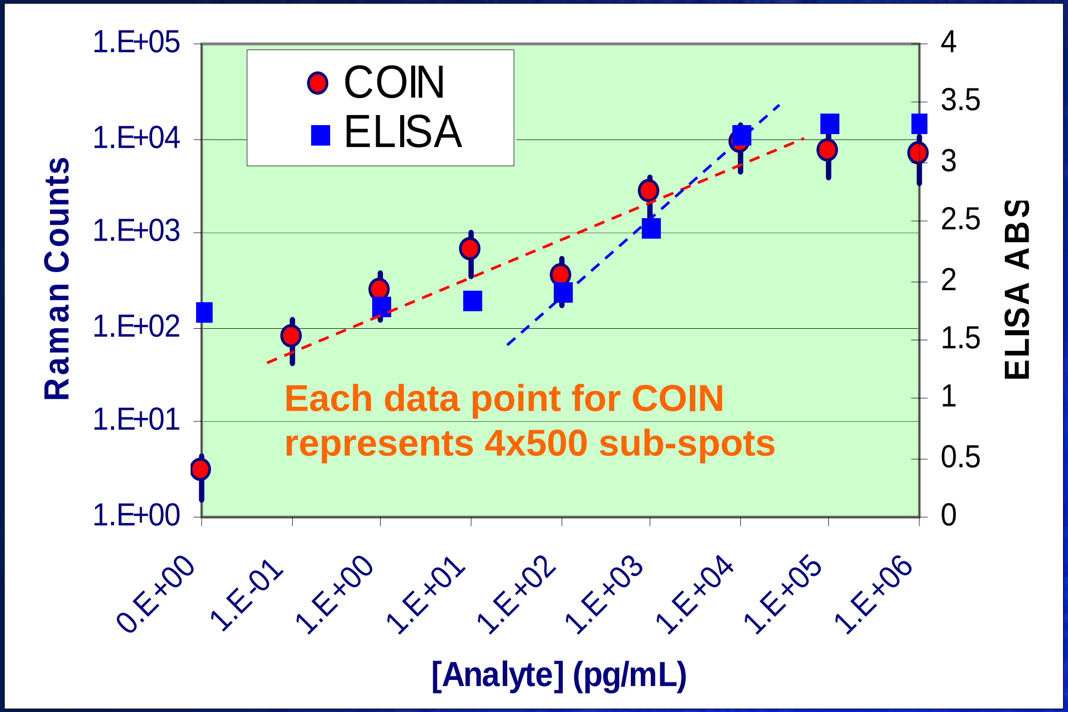


Likely Single Binding Event Detection



COINs Offer High Detection Sensitivity

Sandwich binding assays



	COIN	ELISA
Detection range	5 Logs	2-3 logs
Lower detection limit	0.1 pg/ml	100 pg/ml

Precision Biology Goals

To create fundamental advances in sensor technology, and to work together with the medical community to make it possible to use chips to diagnose disease and improve people's health

RECENT NEWS:

Intel establishes collaboration with the Fred Hutchinson Cancer Research Center

"Advancing Knowledge, Saving Lives"